pendent" — consists of cells more firmly bound with tissue structures, and trypsinization is necessary for their liberation. The role of each of these fractions of stromal precursors in transfer of the microenvironment is a matter for further study.

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AUTORADIOGRAPHIC STUDY OF HORMONE-REGULATED CELL PROLIFERATION IN THE GOLDEN HAMSTER UTERUS DURING POSTNATAL DEVELOPMENT

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Numerous investigations have shown that estrogens stimulate proliferative processes in the uterine epithelium of experimental animals whereas progesterone has an inhibitory action. Conversely, after parenteral injection of estrogen, progesterone stimulates cell proliferation in the stroma of the uterus [1]. Both stimulation and inhibition of proliferation are realized in the tissues of the reproductive tract through interaction between sex hormones and specific receptor proteins. Several workers have found that receptors of both estrogens [9, 12] and progesterone [10] are present in cells of the uterus in rodents at birth, but ability to respond to hormonal stimulation is formed much later [4, 5, 11]. However, ideas on the gradual formation of hormone sensitivity are by no means generally accepted, and there is no unanimity on this problem [3, 8].

The object of this investigation was to study the effect of estrogen, gestagen, and antiestrogen on proliferative activity of epithelial and stromal cells in the golden hamster uterus during postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on 134 female golden hamsters aged from 1 to 20 days. In the experiments of series I (control) intact females were decapitated at the ages of 1, 3, 8, 12, and 20 days. In the experiments of series II the animals were decapitated at the same age 20 h after subcutaneous injection of 10 μg of an oily solution of octestrol. In the experiments of series III hamsters aged 1 and 3 days were decapitated 20 h after subcutaneous injection of 250 μg of an oily solution of 17-hydroxyprogesterone capronate, and animals aged 6, 12, and 20 days were decapitated after two injections of the hormone (44 h after the 1st and 20 h after the 2nd injection). In the experiments of series IV only animals aged 12 days were used. The hamsters were decapitated after two subcutaneous injections of the antiestrogens tamoxifen* (48 and 24 h thereafter).

*The tamoxifen was generaly provided by Dr. A. Todd (Imperial Chemical Industries Ltd., Great Britain).

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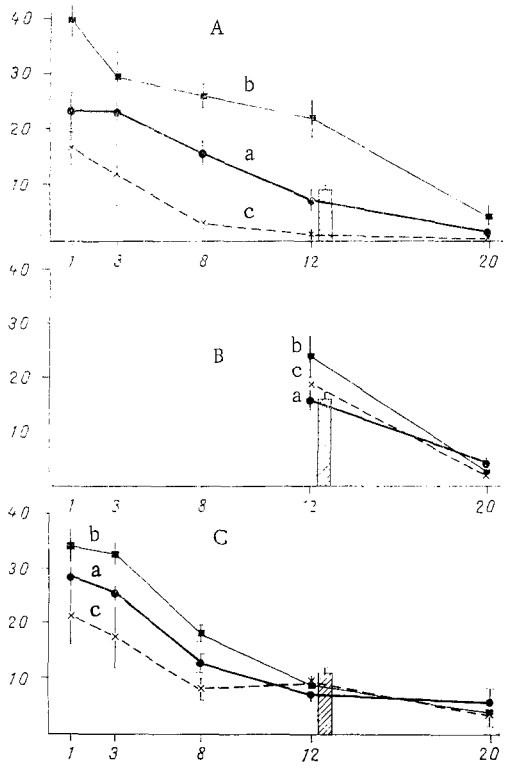

Fig. 1. Dynamics of changes in values of ILN in various structures of golden hamster uterus during normal ontogeny and under the influence of sex hormones. A) Epithelium lining uterine cavity; B) epithelium of uterine glands; C) fibroblasts of uterine stroma. a) Intact animals; b) injection of octestrol; c) injection of 17-hydroxy-progesterone capronate. Shaded columns indicate values of ILN in animals of experiments of series IV (receiving tamoxifen). Abscissa, age of animals (in days); ordinate, value of ILN (in %).

One hour before decapitation the animals were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 0.5 μCi/g body weight. The uterine cornua were fixed in Carnoy's fluid and embedded in paraffin wax. For autoradiographic investigation, sections 4 μ thick were coated with type R photographic emulsion and exposed for 10 days. After development the sections were stained with hematoxylin and eosin. The index of labeled nuclei (ILN) and mitotic index (MI) in per cent were determined by counting 2000 cells of the epithelium lining the uterine cavity, the epithelium of the glands, and fibroblasts of the uterine stroma. The results were subjected to statistical analysis with the aid of the "Elektronika-TZ-16M" specialized computer.

EXPERIMENTAL RESULTS

Investigation of the proliferative activity in the epithelium and stroma of the uterus of the inact animals showed that on the first day after birth values of ILN and MI in both epithelium and stroma were very high. Later, the intensity of proliferation fell, but the decline in proliferative activity of the epithelium did not take place uniformly, on account of the formation of uterine glands. The epithelium of glands formed on the 12th day of life of the hamsters preserved high proliferative activity on account of invagination of folds of the epithelial layer lining the uterine cavity, twice as high as the corresponding values in the epithelium lining the cavity (Fig. 1, Table 1).

TABLE 1. Values of the MI in Epithelium Lining Uterine Cavity, Epithelium of Glands, and Stroma of Uterus in Series of Experiments (M ± m)

Test object	Age of animals, days	Series of experiments: I intact	II (octestrol)	III (17-hydroxyprogesterone capronate)	IV (tamoxifen)
Epithelium of uterine cavity	1	1,48±0,30	3,54±1,01	1,03±0,35	—
	2	1,09±0,14	2,39±0,33	0,28±0,27	—
	8	0,69±0,11	1,61±0,12	0,14±0,14	—
	12	0,66±0,11	1,33±0,23	0,06±0,02	0,77±0,12
	20	0,52±0,27	0,73±0,12	0	—
Epithelium of uterine glands	12	1,27±0,14	1,71±0,31	1,52±0,37	1,17±0,12
		0,73±0,12	0,41±0,12	0,55±0,37	—
Fibroblasts of uterine stroma	1	0,96±0,38	1,72±0,25	0,89±0,36	—
	3	0,76±0,08	0,93±0,14	0,50±0,19	—
	8	0,44±0,11	0,89±0,15	0,43±0,11	—
	12	0,32±0,06	0,61±0,07	0,22±0,08	0,51±0,14
	20	0,32±0,19	0,35±0,11	0,42±0,11	—

The fall in the level of proliferative activity in the uterine tissues of the golden hamsters from the 1st to the 20th days of life did not correspond to the dynamics of the changes in the blood estrogen level during that period [14]. In accordance with the results, proliferative activity of the uterine cells continued to decline, despite the rise in the circulating blood estrogen level taking place in golden hamsters after the 10th day [14]. Meanwhile administration of 10 μg octestrol stimulated proliferation of the cells of the uterine epithelium and stroma after the first day of life of the animal (Fig. 1, Table 1). This result did not agree with those of biochemical investigations on rats [4, 5, 8, 11], which did not reveal any increase in DNA synthesis in response to estrogenic stimulation before the age of 7 days [8] or even 20 days [5, 11] of postnatal development. Meanwhile the results of the present experiments show that the amplitude of the proliferative response to estrogenic stimulation changed considerably depending on the animals' age and the tissue tested. For instance, in the epithelium of the uterine cavity an increase was observed in the amplitude of the rise in ILN in response to injection of estrogen, and it became particularly marked after the formation of the glands on the 12th day of postnatal development. Whereas before the 8th day of the animals' life the increase in the values of ILN as a result of estrogenic stimulation did not exceed 70%, on the 12th and 20th days the mean values of ILN in response to injection of octestrol increased by 192 and 242% respectively. Meanwhile the epithelium of the glands responded to injection of estrogens by a comparatively small increase in proliferative activity on the 12th day of life (the increase in ILN was 51.2%), but on the 20th day this response was absent. The amplitude of the response of the stromal cells decreased with age, and on the 20th day of life the reaction to estrogen was absent.

The results suggest that absence of reactions of the neonatal rodent uterus to estrogens, observed in a number of investigations [4, 5, 1], is connected with the low sensitivity of the biochemical method used by the authors cited to evaluate DNA synthesis. Meanwhile the increase in amplitude of the proliferative response of the epithelial cells of the uterine cavity to estrogenic stimulation with age may indicate that during development the proportion of cells capable of responding to estrogenic stimulation by starting on the mitotic cycle or by a change in duration of the periods of the mitotic cycle increases in the epithelium lining the uterine cavity.

As the data in Fig. 1 and Table 1 show, administration of 17-hydroxyprogesterone capronate inhibited proliferation of the epithelial cells of the uterine cavity at all age groups; just as after administration of octestrol, the amplitude of the response increased with age. Progesterone did not inhibit proliferation in the epithelium of the uterine glands of hamsters aged 12 days. Only in animals aged 20 days was some decrease in ILN found in the epithelium of the glands under the influence of the gestagen (Fig. 1).

The data suggests that the 12th day of life is important both during formation of the morphological structure of the uterus and in the formation of the hormone sensitivity of the uterine cells. Accordingly the animals of this age group were selected in order to

assess the effect of tamoxifen, an antiestrogen, on proliferation; tamoxifen competes with estrogens for the receptor [7, 13] and can thus inhibit proliferative reactions caused by estrogens [6].

The results showed (Fig. 1, Table 1) that tamoxifen caused no changes in proliferative activity in the epithelium of the uterine cavity or in the epithelium of the glands. Conversely, an increase in both MI and ILN was observed in the stroma. The absence of any response of the epithelial cells to the antiestrogens suggests that in the early stages of rodent ontogeny the high level of proliferation in the uterine epithelium is not connected with the influence of endogenous estrogens. This is confirmed by the disparity between the data on the dynamics of changes in the blood estrogen concentrations in hamsters during ontogeny and the changes which we observed in proliferation of the uterine cells.

During postnatal development of the golden hamster, subpopulations of epithelial cells are thus formed in the uterine cavity and glands of the uterus, differing in their sensitivity to estrogens and gestagens, as a result of proliferation and differentiation of the uterine epithelial cells independent of the endogenous estrogen level. This conclusion is in harmony with ideas on the functional heterogeneity of the epithelial cells of the endometrium relative to hormonal influences [2].

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